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=> d que l4
L1           52 SEA FILE=REGISTRY ABB=ON  PLU=ON  YLYQWLGAPVPYPDPL[EX]PRR[EX]VC
                [EX]LNPDCDELADHIGFQEAYRRFYGPV/SQSP
L2          566 SEA FILE=REGISTRY ABB=ON  PLU=ON  GLA/NTE
L3           42 SEA FILE=REGISTRY ABB=ON  PLU=ON  L1 AND L2
L4            7 SEA FILE=CAPLUS ABB=ON  PLU=ON  L3
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(E)

[EX] = Glutamic Acid or Uncommon or Unspecified AA (one letter code) (X)

/SQSP = Subsequence search field

Gla = 3 letter code for γ-carboxy glutamic acid

/NTE = Annotation Field

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=> D IBIB ABS 1-7

L4   ANSWER 1 OF 7  CAPLUS  COPYRIGHT 2002 ACS
ACCESSION NUMBER:         1997:684556  CAPLUS
DOCUMENT NUMBER:          127:345337
TITLE:                    Anti-Glu17-osteocalcin antibody
INVENTOR(S):              Sakakibara, Shunpei; Kimura, Terutoshi; Morimoto,
                          Shigeto
PATENT ASSIGNEE(S):       Eisai Co., Ltd., Japan; Sakakibara, Shunpei; Kimura,
                          Terutoshi; Morimoto, Shigeto
SOURCE:                   PCT Int. Appl., 28 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 Japanese
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.        KIND  DATE           APPLICATION NO.  DATE
     ---------------   ----  --------       ---------------  --------
     WO 9738309         A1   19971016       WO 1997-JP1246   19970410
         W:  US
         RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
     JP 09329600        A2   19971222       JP 1997-43331    19970227
     EP 834740          A1   19980408       EP 1997-915706   19970410
         R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI
PRIORITY APPLN. INFO.:                  JP 1996-88608       19960410
                                        JP 1997-43331       19970227
                                        WO 1997-JP1246      19970410
AB   An antibody capable of discriminating Glu17-osteocalcin from osteocalcin.
     An anti-Glu17-osteocalcin antibody or fragments thereof, characterized by
     being specifically bonded to Glu17-osteocalcin having a Glu residue at the
     17-position or to osteocalcin fragments contg. a Glu residue at the
     17-position.  Antigenic peptide of osteocalcin having Glu17 replaced by
     .gamma.-carboxyglutaminc acid was synthesized, conjugated with albumin or
     ovalbumin for raising the disclosed monoclonal IgGs.  Labeled antibody was
     prepd. and used for detecting Glu17-osteocalcin in human blood plasma and
     for predicting risk factors for or diagnosing and treating postmenopausal
     osteoporosis and other bone loss diseases.

L4   ANSWER 2 OF 7  CAPLUS  COPYRIGHT 2002 ACS
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ACCESSION NUMBER: 1996:574837 CAPLUS
DOCUMENT NUMBER: 125:266837
TITLE: Purification and characterization of recombinant osteocalcin fusion protein expressed in Escherichia coli
AUTHOR(S): Kaekoenen, Sanna-Maria; Hellman, Jukka; Pettersson, Kim; Loevgren, Timo; Karp, Matti
CORPORATE SOURCE: Dep. of Biotechnology, Univ. of Turku, Turku, FIN-20520, Finland
SOURCE: Protein Expression and Purification (1996), 8(2), 137-144
CODEN: PEXPEJ; ISSN: 1046-5928
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Human osteocalcin (hOC) is a 49-amino-acid peptide produced mainly by bone osteoblasts. The amt. of hOC in the circulation reflects the status of bone metab. and it is used to monitor various bone-related diseases. The aim of this study was to produce recombinant human osteocalcin (rhOC) in Escherichia coli and use it for designing new osteocalcin fluorescence immunoassays. Recombinant DNA technol. was used to fuse synthetic hOC coding sequences to an affinity handle system based on glutathione S-transferase (GST) gene. GST-rhOC fusion protein was produced in a bacterial intracellular expression system mainly in a sol. form. The affinity-purified fusion protein was cleaved with activated protease factor X releasing the rhOC portion. The structure of rhOC was confirmed by mass spectrometry and amino acid sequencing. The fusion protein and its proteolytic cleavage product proved to be immunoreactive as shown by Western blotting anal. and by a new osteocalcin immunoassay based on time-resolved fluorescence. When osteocalcin was tested for its ability to bind to hydroxyapatite, there were no differences between the recombinant forms and native human osteocalcin purified from bone, suggesting that the Gla residues might be important only in oriented high-affinity binding.

L4 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:40403 CAPLUS
DOCUMENT NUMBER: 122:161286
TITLE: Synthesis of human osteocalcins: .gamma.-carboxyglutamic acid at position 17 is essential for a calcium-dependent conformational transition
AUTHOR(S): Nakao, M.; Nishiuchi, Y.; Nakata, M.; Kumura, T.; Sakakibara S.
CORPORATE SOURCE: Peptide Inst., Protein Research Foundation, Osaka, Japan
SOURCE: Peptide Research (1994), 7(4), 171-4
CODEN: PEREEO; ISSN: 1040-5704
DOCUMENT TYPE: Journal
LANGUAGE: English
GI

1
Tyr-Leu-Tyr-Gln-Trp-Leu-Gly-Ala-Pro-Val-Pro-Tyr-Pro-Asp-Pro-

17 21 24
Leu-Glu/Gla-Pro-Arg-Arg-Gla-Val-Cys-Gla-Leu-Asn-Pro-Asp-Cys-

Asp-Glu-Leu-Ala-Asp-His-Ile-Gly-Phe-Gln-Glu-Ala-Tyr-Arg-Arg-

49
Phe-Tyr-Gly-Pro-Val

I

AB Human osteocalcins with amino acid sequence I (Gla = .gamma.-carboxyglutamic acid) having two (positions 21 and 24) or three (positions 17, 21 and 24) .gamma.-carboxyglutamic acids (Gla) were synthesized in soln. employing both the Boc strategy and the HF procedure for the final deprotection. During synthesis, the .gamma.,.gamma.-dicarboxyl functional groups of the Gla residues were protected by the cyclohexyl group. The identity of the synthetic peptides was confirmed by amino acid anal., mass spectrometric measurement and peptide mapping. CD measurement showed that the conformation of osteocalcin contg. three Gla residues dramatically changed on addn. of calcium ions, wheres the peptide contg. glutamic acid at position 17 did not. These findings clearly show that the Gla residue at position 17 is essential for a calcium-dependent conformational transition of osteocalcin.

L4 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:436189 CAPLUS
DOCUMENT NUMBER: 121:36189
TITLE: Solid-phase synthesis of human osteocalcin by using a .gamma.-carboxyglutamic acid derivative
AUTHOR(S): Kurihara, Takashi; Taniyama, Eiji; Hane, Motomu; Saito, Takao; Hirose, Sachio; Ohashi, Shinichi
CORPORATE SOURCE: Tsukuba Med. Dev. Group, Mitsubishi Petrochem. Co. Ltd., Inashiki, Japan
SOURCE: International Journal of Peptide & Protein Research (1994), 43(4), 367-73
CODEN: IJPPC3; ISSN: 0367-8377
DOCUMENT TYPE: Journal
LANGUAGE: English
OTHER SOURCE(S): CASREACT 121:36189
AB Human osteocalcin, also called bone Gla protein (BGP), consisting of 49 amino acids with two to three .gamma.-carboxyglutamate residues, was chem. synthesized for the first time by a novel solid-phase peptide synthesis. Protected .gamma.-carboxyglutamic acid (Gla) deriv. Boc-L-NHCH(CO2H)CH2CH(CO2cHex)2 (L-I; Boc = Me3CO2C, cHex = cyclohexyl) was designed, prepd. and utilized as a monomeric compd. and proven to be useful for the solid-phase peptide synthesis of human osteocalcin. The synthesis and optical resoln. of DL-I are described, followed by the synthesis and characterization of Gla17-human osteocalcin.

L4 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:449922 CAPLUS
DOCUMENT NUMBER: 119:49922
TITLE: Preparation of human osteocalcin analogs

INVENTOR(S): Eguchi, Hiroshi; Nakamoto, Tadakatsu; Pponda, Hitomi; Kubota, Takaaki; Okada, Masahiro; Hosoda, Kenji; Imaizumi, Atsushi
PATENT ASSIGNEE(S): Teijin Ltd, Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 10 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 05032697	A2	19930209	JP 1991-213251	19910731

GI

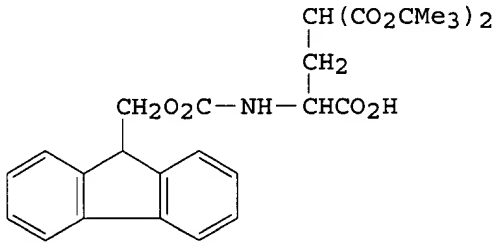
H-Tyr-Leu-Tyr-Gln-Trp-Leu-Gly-Ala-Pro-Val-Pro-Tyr-
1 5 10

Pro-Asp-Pro-Leu-X-Pro-Arg-Arg-Gla-Val-Cys-Gla-
15 20

Leu-Asn-Pro-Asp-Cys-Asp-Glu-Leu-Ala-Asp-His-Ile-
25 30 35

Gly-Phe-Gln-Glu-Ala-Tyr-Arg-Arg-Phe-Tyr-Gly-Pro-
45

Val-OH I



II

AB 21,24Gla- and 17,21,24Gla-human osteocalcin (I; X = Glu, Gla; Gla = .gamma.-carboxyglutamic acid residue), useful as std. substances for detn. of human osteocalcin and for the treatment of bone metabolic diseases, are prepd. I were prepd. by the solid phase method using a peptide synthesizer 431A (Applied Biosystems Inc.) and a protected L-.gamma.-carboxyglutamic acid (II) (prepn. given). I showed the same immunochem. reaction with that of the natural human osteocalcin.

L4 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1992:56694 CAPLUS
DOCUMENT NUMBER: 116:56694
TITLE: Serum BGP concentration in patients with osteogenesis imperfecta and achondroplasia. Comparative study between RIA and IRMA methods

AUTHOR(S): Yamamoto, Takehisa; Yamaoka, Kanji; Kurose, Hirofumi; Okada, Shintarou; Inoue, Masaru; Tanaka, Hiroyuki; Seino, Yoshiki
CORPORATE SOURCE: Sch. Med., Osaka Univ., Osaka, 530, Japan
SOURCE: Horumon to Rinsho (1991), 39(10), 1031-4
CODEN: HORIAE; ISSN: 0045-7167
DOCUMENT TYPE: Journal
LANGUAGE: Japanese

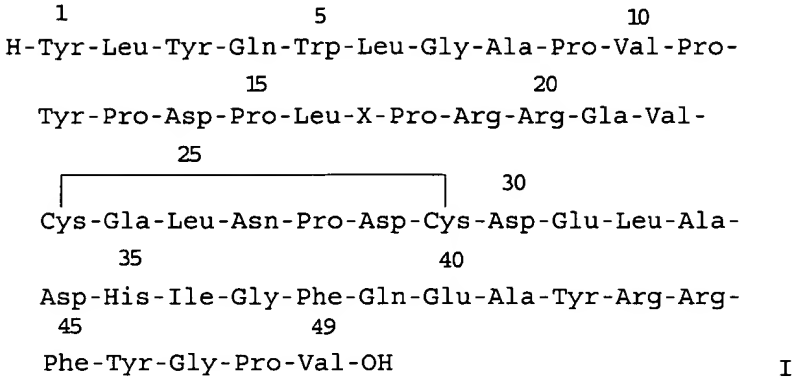
AB Serum osteocalcin (BGP) in patients with osteogenesis imperfecta and achondroplasia was detd. by using RIA (RIA) and immunoradiometric assay (IRMA) methods. Serum alk. phosphatase (ALP) tended to increase in both diseases. Serum BGP concn. in achondroplasia had the same value in both RIA and IRMA methods. However, patients with osteogenesis imperfecta were classified into 2 groups, group I showed higher serum BGP levels by IRMA method than that by RIA and group II showed the same value in both RIA and IRMA methods. Serum ALP and urine hydroxyproline/creatinine ratio were higher in group I than in group II.

L4 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1991:680565 CAPLUS
DOCUMENT NUMBER: 115:280565
TITLE: Method for preparing human osteocalcin
INVENTOR(S): Kurihara, Takashi
PATENT ASSIGNEE(S): Mitsubishi Petrochemical Co., Ltd., Japan
SOURCE: Eur. Pat. Appl., 29 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 418617	A1	19910327	EP 1990-116754	19900831
EP 418617	B1	19941109		
R: BE, DE, FR, GB, IT				
JP 03090100	A2	19910416	JP 1989-223203	19890831
JP 07068271	B4	19950726		
US 5164483	A	19921117	US 1990-575639	19900831
US 5258545	A	19931102	US 1992-886815	19920522

PRIORITY APPLN. INFO.: JP 1989-223203 19890831
US 1990-575639 19900831

GI



I

AB Human osteocalcin I [X = Glu, Gla (Gla = .gamma.-carboxyglutamic acid residue)] and salts were prepd. via solid phase methods comprising introduction of the .gamma.-carboxyglutamic acid residue by use of protected L-.gamma.-carboxyglutamic acid Boc-NHCH[CH2CH(CO2R)]2 CO2H (II) (R = cyclopentyl, cyclohexyl, cycloheptyl). Thus, Boc-Ser-OH was converted to the benzyl ester, which was tosylated and treated with Et2NH to give Boc-NHC(:CH2)CO2Bzl. To this was added dicyclohexyl malonate [prepn. from CH2(CO2Me)2 given] in the presence of NaH to give the Michael addn. product, which was hydrogenated to give II (R = cyclohexyl) (III). The L-isomer of III was used in the synthesis of I (X = Gla) using the appropriate (protected) amino acids and a phenylacetamidomethyl resin. A measuring system was developed to quantify human osteocalcin in blood serum.